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Communication

Design and Synthesis of Metabolically Stable tRNA Synthetase Inhibitors Derived from Cladosporin

Dr. Marion Rusch,[a] Arnaud Thevenon,[a,b] Dr. Dominic Hoepfner,[a] Thomas Aust,[a] Christian Studer,[a] Maude Patoor,[a] Prof. Patrick Rollin,[c] Madeleine Livendahl,[a] Dr. Beatrice Ranieri,[a] Esther Schmitt,[a] Carsten Spanka,[a] Prof. Dr. Karl Gademann 0000-0003-3053-0689,[d] and Dr. Laure^^C. Bouchez 0000-0003-4583-6760*[a]

[a] *NIBR--Novartis Institute for Biomedical Research*
Fabrikstrasse 22-1.051.17, 4054 Basel (Switzerland)
E-mail: laure.bouchez@novartis.com

[b] *Department of Chemistry, Imperial College Oxford University--remove, OK?*
London SW7 2AZ (UK)

[c] *Institut de Chimie Organique et Analytique (ICOA)*
UMR7311, Université d'Orléans
45100 Orléans (France)

[d] *University of Zürich, Department of Chemistry*
Winterthurerstrasse 190, 8057 Zürich (Switzerland)

Supporting information and the ORCID identification numbers for the authors of this article can be found under https://doi.org/10.1002/cbic.201800587.

Ring in the changes: Selective and specific inhibitors of *Plasmodium falciparum* tRNA synthetases represent promising therapeutic antimalarial avenues. Chemogenomic profiling of a focused library allowed the identification of a simplified cladosporin analogue, comprising an improved potency, a superior metabolic stability to that of its natural parent, and retaining its selectivity towards the human enzyme.

Synthesis of a simplified analogue of the natural product cladosporin for #antimalarial #drugdiscovery (Bouchez @NovartisScience) @karlgademann @uzh_science

antimalarial agents
drug discovery
inhibitors
natural products
structure--activity relationships

Selective and specific inhibitors of *Plasmodium falciparum* lysyl-tRNA synthetase represent promising therapeutic antimalarial avenues. Cladosporin was identified as a potent *P.^^falciparum* lysyl-tRNA synthetase inhibitor, with an activity against parasite lysyl-tRNA synthetase >100-fold more potent than that of the activity registered against the human enzyme. Despite its compelling activity, cladosporin exhibits poor oral bioavailability; a critical requirement for antimalarial drugs. Thus, the quest to develop metabolically stable cladosporin-derived analogues, while retaining similar selectivity and potency to that of the natural compound, has begun. Chemogenomic profiling of a designed library allowed an entirely innovative structure--activity relationship study to be initiated; this shed light on structural evidence of a privileged scaffold with a unique activity against tRNA synthetases.

Every year, malaria is responsible for over 500^^million cases requiring hospitalization. The rapid spread of antimalarial drug resistance limits the availability of armamentarium to treat these patients. Therefore, we are in urgent need of the next-generation drug that will shift the path towards increasing sustainability of treatment regimens and delaying the emergence and spread of drug resistance, as far as possible.[1--3]

Cladosporin, a fungal secondary metabolite, was identified during the course of a screening campaign run against both *Plasmodium falciparum* blood- and liver-stage proliferation.[4] Intrigued by its potent activity, several research groups embarked on its biosynthesis and total synthesis to provide a path to advance it in the antimalarial drug pipeline.[5] Unpredictably, despite its enticing nanomolar antiparasitic activity, cladosporin regressed very quickly to a tool compound class that could no longer be progressed due to its weak metabolic stability and high clearance in vivo.[6]

In particular, for antimalarial drugs, oral bioavailability is critical because affected countries often lack the infrastructure for complex therapies. Cladosporin showed a very high clearance in both human and mouse models (subtype ER(human) 0.64^^CL_{int}(μL^min$^{<M->1}$^mg$^{<M->1}$)=46.926; ER(mouse) 0.95^^CL_{int}(μL^min$^{<M->1}$^mg$^{<M->1}$=425)).[6] To reveal potential liabilities that could impair further its development as a drug candidate, cladosporin was also incubated in vitro with mouse liver microsomes. Each sample was analyzed by means of capillary-HPLC/$MS^{(n)}$ and the proposed chemical structures were in accordance with accurate mass measurement of the corresponding protonated molecular ions. To our surprise, cladosporin was barely detectable. The only metabolic pathway that could be detected was the glucuronidation of cladosporin. More in-depth studies revealed three major fragmentations that helped to identify the structural Achilles' heels responsible for fast clearance in both human and mouse. The weak points, including the three major fragmentations that were observed, are highlighted in Scheme^^1<schr1>. They serve in the elaboration of our lead optimization approach.

The apparent structural simplicity of the cladosporin template motivated us to propose and synthesize a series of cladosporin-inspired analogues. The rationale for the design of our focused library was based on structural weaknesses revealed by our metabolic evaluation and in silico docking study based on cladosporin-lysyl-tRNA synthetase (KRS1) cocrystal structures published recently (Scheme^^1<xschr1>, Figure^^1<figr1> and the Supporting Information).[4]

Indeed, the crystal structure can also be interrogated in stereochemical and structural terms. Cladosporin binds in the ATP-binding pocket of the lysyl-tRNA synthetase. The major interactions appear to be a hydrogen bond with Glu332 and the π/cation "cage" formed from adjacent aromatic and charged residues. The lactone ring interacts with the Arg559 main chain through structural water and must remain unchanged to retain the principal electrostatic interactions. Under these premises, we presumed that meaningful structure--activity relationship (SAR) studies should focus on the elucidation of the role of hydroxy groups, the tetrahydropyran frame, and carbon C4 of the isochromenone core (Figure^^2<figr2>). Any such structural editing, however, is difficult to achieve if one starts from the natural products

themselves; de novo synthesis seems to be much more appropriate to garner relevant insights. Because our group was previously involved in the synthesis of Agrimonolide,[5] we felt that we were in a strong position to venture into such an endeavor. Indeed, the variety of accumulated building blocks, starting from resorcinol to the *m*-methylaniline unit, allowed for the rapid generation of novel compounds with altered properties and unprecedented biological activities.

Three main strategies were devised and executed in parallel to generate a focused library that was subsequently assessed for its activity and metabolic stability. Although not all steps were optimized for each individual analogue prepared during this campaign, the first route turned out to be efficient and reproducible in most cases. Starting from commercially available 3,5-methoxybromobenzene (**1**) and from *tert*-butyloxycarbonyl (*N*-Boc)-protected-3-bromo-5-methylaniline (**11**), both C<C->Br bonds are activated under Stille coupling conditions in the presence of tetrakis(triphenylphosphine)palladium and LiCl to give the corresponding allyl derivatives **2** and **12** in 83 and 68^%, respectively. Next, compounds **2** and **12** are cross-reacted with allylcyclopentane, allylcyclohexane, and **13**[5] in the presence of the Grubbs II catalyst, and subjected to a Sharpless dihydroxylation with AD-mix-α to provide three new diol intermediates, **3**, **4**, and **14**, as single enantiomers and in relatively good yield over two steps (29, 43, and 26^%, respectively).[7] After reaction with thiocarbonyl diimidazole in toluene at reflux, the corresponding thiocarbonates **5**, **6**, and **15** could be isolated. Further treatment with a mixture of Bu_3SnH and azobisisobutyronitrile (AIBN),[8] allows for selective deoxygenation and the generation of the corresponding monoalcohols in a regioselective manner. Further treatment with trimethyl orthoformate under acidic conditions, followed by Jones oxidation, afforded the three corresponding lactones **7**, **8**, and **16**.[9] Final deprotection in the presence of BBr_3 yielded the first analogues, **9** and **10**, in 60 and 73^% yield, respectively. Alternatively, lactone **16** was treated with a solution of trifluoroacetic acid (TFA) and subsequently hydrogenated to provide aniline analogue **17** in 46^% yield over two steps. Satisfyingly, this first generation of analogues allowed for the facile preparation of over 30 analogues, in a 9-step procedure, with an overall yield of roughly 8^% (Scheme^^2<schr2>; see the Supporting Information for additional examples and respective data).

For our second approach, encouraged by the docking study and potential shape complementarity, we explored analogues containing a stereocontrolled tetrahydropyran core (Scheme^^3<schr3>). The synthesis of dihydroxyisocoumarins **25^a** and **25^b** commences with a similar strategy. Compound **1** is converted into 2-bromo-4,6-dimethoxybenzaldehyde under standard Vilsmeier--Haack conditions.[10] $KMnO_4$ oxidation[11] and methyl iodide treatment in the presence of K_2CO_3 gives the desired methyl ester **18**. Palladium-catalyzed Stille coupling with allytributylstannane affords allylic intermediate **19** (92^% yield), which is subjected to the Lemieux--Johnson oxidative cleavage,[12] to provide smoothly aldehyde **20**. Subsequent enantioselective Brown allylation[13] delivers chiral homoallylic alcohol **21**, which partially undergoes spontaneous intramolecular esterification with the neighboring ester functionality. Final cyclization of the remaining acyclic material is promoted by Amberlyst^^15 catalyst,[14] leading to allyl-modified isochromanone **22** in 60^% yield over two steps. This compound is finally exposed to a catalytic amount of Grubbs^^II complex (5^%) in benzene at 45^°C to perform a crosscoupling metathesis reaction with the homoallylic alcohol (*S*)-(<M+>)-hex-5-en-2-ol, leading to a remarkably clean formation of *E*-olefin **23** in 64^% yield. At this stage, despite the fact that we were seemingly only two routine operations away from final lactone **25**, we could not force the complete stereoselectivity of the ring-closure step. Moreover, given our interest in accessing both diastereoisomers, no particular effort was further dedicated to guide the stereoselectivity specifically for ring closure. Hence, the strategy continues with an iodine-mediated cyclization that allows the rapid generation of the C5-membered ring system. The iodo intermediate formed is engaged directly in a halogen--hydride exchange reaction by employing Bu_3SnH/AIBN to yield a mixture of two diastereoisomers, **24^a** and **24^b**, which are highly inseparable. Only after proceeding to their separation through reversed-phase chiral HPLC were **24^a** and **24^b** isolated with 99^% diastereomeric excess (*de*).[15] Ultimate deprotection under Maier's conditions (AlI_3, TBAI, and phloroglucinol)[16] leads to lactones **25^a** and **25^b,** which could be unambiguously characterized with a fully assigned configuration (see the Supporting Information).

Our third strategy resembles a small pilot synthesis, on a simplified set of analogues. A representative case is outlined in Scheme^^4<schr4>. In fact, the presence of the

isocoumarin ring was known to be vital, but some structural modifications were allowed by the "π cage" formed with the aromatic residues. Inspired by previous findings from a set of compounds generated by biotransformation, we proposed to introduce a strategic unsaturation within the isocoumarin core, as illustrated by compound **28**.[17a]

As shown in Scheme^^4<xschr4>, our pivotal starting material, methyl 2-bromo-4,6-dimethoxybenzoate (**18**) is subsequently engaged in a Sonogashira coupling reaction with prop-2-yn-1-ylcyclohexane (**26**) in the presence of a catalytic amount of copper iodide and trimethylamine, leading to alkynylaryl ester **27** in 80^% yield. $FeCl_3$-mediated cyclization of **27** selectively generated the expected six-membered ring product, which was subjected to BBr_3 treatment, to afford analogue **28** ready for biological evaluation (Scheme^^4<xschr4>).[18]

With all of these analogues in hand, we initiated a profiling campaign to assess their respective biological activity/selectivity for different tRNA synthetases/lysyl, threonine and phenylalanine, as well as their metabolic stability. Follow-up analysis, by means of the haploinsufficiency profiling (HIP) method and genetic mutational profiling, [19] confirmed that compounds **9**, **10**, and **28** were still on target, and the overall lysyl-tRNA binding mode was unchanged (see the Supporting Information).[20] This key assay allowed us not only to assess activity and selectivity in a cell-based fashion, but also to verify that the tested derivatives had the potential to penetrate even thick biological cell walls and membranes; an important requirement for potential anti-infectives.

Critically, although presenting a relatively close structural similarity to that of cladosporin, compounds **9** and **10** ($IC_{50,\,p.f.\text{LysRS}}$=0.3; 0.2^^μM) were found to be several-fold less potent and less selective than the parent compound cladosporin (Table^^1<tabr1>). Compounds **25^a** and **25^b** did not present any activity ($IC_{50,\,p.f.\text{LysRS}}$>10^^μM). HIP analysis of compounds **17** and **29** (obtained from commercial sources) gave a most surprising outcome because their respective activity revealed that the observed cell-based activity was not elicited by inhibition of lysyl-tRNA synthetase, but rather threonyl- and phenylalanyl-tRNA synthetase, respectively (see the Supporting Information).

Finally, the metabolic stability was assessed for selected compounds with interesting biological activities. The results are reported in Table^^2<tabr2> as a measure of intrinsic clearance (CL int), half-life ($t_{1/2}$), and ER in humans. As aligned with their low potency and selectivity, structural modifications conferred to compounds **9** and **10** did not address the metabolic stability aspect and were as weak as cladosporin. Astonishingly, the seemingly minor modifications proposed earlier payed off because compound **28** turned out to be significantly more potent than cladosporin itself, while conserving its selectivity and presenting a reasonable metabolic stability (Table^^2<xtabr2>). Notably, because the glucuronidated product of **28** could not be observed, we did not engage any further campaign to modify any of the hydroxy groups carried by the aromatic moiety of the molecule.

In conclusion, we have successfully identified a simplified cladosporin analogue that presents not only improved potency and metabolic stability, compared with that of its natural parent, but retains selectivity towards the human enzyme. Gratifyingly, our screening campaign has led to the identification of two additional fungal active drugs that are selective inhibitors of threonyl- and phenylalanyl-tRNA synthetase, and thus, expanding the biological space modulated by this isocoumarin-derived scaffold. Additionally, as reported in the previous publication,[17a] an additional biotransformation-based approach resulted in the discovery of a set of active hydroxylated cladosporin analogues, which targeted specifically the lysyl-tRNA synthetase. This discovery represents a real breakthrough because it suggests that such a natural product derived structure could provide a privileged scaffold to inhibit synthetases beyond lysyl-tRNA synthetase.

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Conflict of Interest

The authors declare no conflict of interest.

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Scheme^^1 Metabolic liabilities and proposed modifications for the cladosporin core structure.

Scheme^^2 First-generation synthesis for the preparation of **9**, **10**, and **17**. a)^^Vinyl-Bu_3Sn, $[Pd(PPh_3)_4]$ (2^^mol^%), Cs_2CO_3, DMF, 100^°C, 16^^h; **2**: 83^%, **12**: 68^%; b)^^allylcyclopentane or allylcyclohexane, Grubbs II cat. (10^^mol^%), CH_2Cl_2, reflux, 16--30^^h; c)^^AD-mix-α, $MeSO_2NH_2$, *t*BuOH/H_2O (1:1), 0^°C, 2--5^^days; **3**: 29^%, **4**: 43^%, **14**: 26^% (over two steps); d)^^thiocarbonyldiimidazole, 1,2-dichloroethane (DCE), reflux, 4--6^^h; **5**: 36^%, **6**: 78^%, **15**: 50^%; e)^^Bu_3SnH, AIBN, toluene, reflux, 4^^h;

f)^^$CH(OMe)_3$, *p*-toluenesulfonic acid (*p*-TSA), CH_2Cl_2, RT, 2^^h; g)^^Jones reagent, acetone, 0^°C to RT, 1^^h; **7**: 42^%, **8**: 64^%, **16**: 27^% (over three steps); h)^^BBr_3, CH_2Cl_2, 0^°C to RT, 5^^days; **9**: 60^%, **10**: 73^%; i)^^TFA, CH_2Cl_2, RT, 16^^h; j)^^10^% Pd/C, H_2, EtOAc, RT, 16^^h; **17**: 46^% (over two steps).

Scheme^^3 Second route proposed, leading to the isolation of **25^a** and **25^b**. a)^^$POCl_3$, DMF, 100^°C, 4^^h, 91^%, b)^^$KMnO_4$, H_2O, 75^°C, 4^^h, 64^%, c)^^MeI, K_2CO_3, DMF, RT, 3^^h, 95^%. d)^^[$Pd(PPh_3)_4$], LiCl, allyltributylstanane, DMF, 100^°C, 24^^h, 92^%; e)^^OsO_4, $NaIO_4$, dioxane/water (3:1), RT, 5^^h, 62^%; f)^^(<M+>)-Ipc_2B(allyl)borane ((<M+>)-*B*-allyldiisopinocampheylborane), Et_2O, <M->78^°C to RT, 2^^h; g)^^Amberlyst^^15, CH_2Cl_2, RT, 2^^days, 60^% (yield over two steps); h)^^(*S*)-(<M+>)-5-hexen-2-ol, Grubbs II cat. (5^^mol^%), benzene, 45^°C, 6^^h, 64^%; i)^^I_2, $NaHCO_3$, toluene, <M->78^°C RT, 18^^h; then Bu_3SnH, AIBN, benzene, 80^°C; **24^a**: 21^%, **24^b**: 21^% (57^% yield over two steps and 42^% after chiral separation); j)^^Al, I_2, tetrabutylammonium iodide (TBAI), phloroglucinol, benzene, 30^^min, 5^°C; **25^a**: 58^%, **25^b**: 42^%.

Scheme^^4 Third route for a simplified approach to cladosporin analogue **28**. a)^^[$Pd(PPh_3)_4$], CuI, NEt_3, DMF, 80^%; b)^^$FeCl_3$, CH_2Cl_2, RT, 18^^h, 40^%; c)^^BBr_3, CH_2Cl_2, RT, 8^^h, 55^%.

Figure^^1 Pertinent part of the crystal structure of cladosporin/ KRS1: cladosporin binds in the adenosine triphosphate (ATP)-binding pocket of the lysyl-tRNA synthetase acylation domain.

Figure^^2 A)^^Major interactions between cladosporin and KSR1. B)^^Structural modifications relative to the parent natural product cladosporin are colored; C4 of the isochromenone core is specifically annotated (4).

Table^^1 <w=1>IC_{50} values for the inhibitory activity of the more potent cladosporin analogues in the two yeast species *P.^^falciparum* versus human. Values compared with the parent compound cladosporin.

Compound	IC_{50} [μM] *S.^^cerevisiae* with		HIP target hypothesis
	human Lys.R.S.	*P.^^falciparum* Lys.R.S.	
cladosporin	100	0.05	lysyl-tRNA synth.
9	30	0.3	lysyl-tRNA synth.
10	15	0.2	lysyl-tRNA synth.
17	180	40	phenylalanyl-tRNA synth.
25^a	>100	>100	n.d.[a]
25^b	>100	>100	n.d.[a]
28	10	0.02	lysyl-tRNA synth.
<forr1>	40	40	threonyl-tRNA synth.

[a]^^n.d.: not determined.

Table^^2 <w=1>Metabolic stability assessment of the key synthetic analogues relative to the parent compound cladosporin.

Compound	Subtype	$t_{½}$ [min]	Hepatic extraction [%]	CL int [μL^min<M->1>^mg<M->1>]
cladosporin	human	269	12	6.9
9	human	370	12	3.2
10	human	405	12	3.4
17	human	13	80	104

28	human	14	79.5	101
